A RESEARCH PROPOSAL

‘‘FORMULATION AND EVALUATION OF TOPICAL DOSAGE FORM USINGSHAT–DHAUT GHRITA FOR ITS PHARMACOLOGICAL ACTIVITY’’

Submitted

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1. INTRODUCTION

The word Ayurveda is derived from the Sanskrit word ‘Ayus’ (all aspects of life from birth to death) and ‘Veda’ (knowledge or science) science of long life. Ayurveda, the most ancient system of traditional medicine of the world, practiced in Indian subcontinent since 5000 BC. Ayurveda is a holistic approach towards life, health and disease management through medicinal herbs, minerals, diet, lifestyle and spirituality [1]. Herbs and products containing herbs have been in trade and commerce and are currently used for a variety of purposes [2]. The WHO defines an herb as being fresh or dried, fragmented or powdered plant [3]. Herbal medicines are plant derived materials and preparations with therapeutic or other human health benefits, which contain either raw or processed ingredients from one or more plants, inorganic materials or animal origin. Herbal medicine preparations are developed and created drugs by the modern pharmaceutical industry. Nowadays, they are manufactured and sold most widely on the pharmaceutical market for curing diseases and promoting public health in India, which can be used in this crude state or further processed and formulated to become the final herbal product [4].

Ghrita

Ghrita in Sanskrit also called as clarified butter or ghee, has been used for more than 5,000 years throughout the Indian subcontinent and has been an inevitable part of diet in the Indian cuisine. Apart from the incredible taste it delivers to the food items, it has been traditionally associated with various health benefits in Charaka, the most ancient written text of Ayurveda dating to second century B.C., as promoting strength and longevity, providing protection from various diseases, promoting appetite and digestion, increasing cognition, providing lubrication to the various parts of the body, etc. On the other hand, the researches of modern medical science have associated Ghrita to a number of diseases such as

cardiovascular diseases (CVDs), ischemic heart disease, obesity, type 2 diabetes mellitus, stroke, overall increase in mortality etc [5].

Mode of Action of Ghrita

Ghee is mainly lipophilic in nature and this action of ghee facilitates transportation of ingredients of formulation to target organ and final delivery inside the cell, because cell membrane also contains lipids. Ghrita contains poly unsaturated fatty acids (PUFA) i.e., DHA- Omega 3 long chained PUFA in fair amounts. Intake of PUFA in moderate levels is found to improve levels of acetyl choline thus reducing chances of dementia [6]. Lipophilic nature of Ghrita facilitates entry of the formulation into the cell and its delivery to the mitochondrion, microsome and nuclear membrane. When herbs are processed with Ghrita, their activity and utility get potentiated to many folds. If drugs given in the form of Ghee which is a fat are rapidly absorbed and distributed in the target areas of the body like the nervous system in this case. Distribution of drug in blood is influenced by its Lipid solubility, Ionization & differences in blood flow. A Water-soluble drug is usually distributed in the extracellular spaces and it may not readily diffuse in to CSF and other body cavities. While the Lipid soluble drugs get rapidly distributed to intra and extracellular spaces [7].

Benefits of Ghrita

As described in Ayurveda Charaka, the most ancient written text of Ayurveda dating to second century B.C., has inscribed the benefits and indications of Ghrita as the best amongst all unctuous substances or fats. It promotes memory, intellect, digestive capacity, reproductive capacity, fatty tissue, and Oja (the vital essence of all tissue crucial for life and immunity). Ghrita has been described to alleviate Vata and Pitta and increase Kapha (Vata, Pitta and Kapha are three bodily humors that make up one's constitution according to Ayurveda and working in coherence and equilibrium are responsible for all the functions of the body.

Ghrita is also described to alleviate poisons, insanity, phthisis, fevers, epilepsies, syncope) and chronic pains occurring in the genital tracts, ears and head. Vagabhatta, the author of the classical text Ashtanga Hridaya and Ashtanga Sangraha, has additionally described Ghrita to be also beneficial for patients suffering from tumors, sinuses, ulcers and worm's diseases. Ghrita is described to have unctuous, penetrating property along with being light in digestion as well as have sweet taste, cold potency and sweet post digestion effect. All these properties make it an excellent vehicle for lubrication, both for internal consumption as well as for external application.

Ghrita has been described as the most unique substance in the fact that it possesses the effect of Sanskarohigunantaradhana (It adopts on the properties of herbs and substances with which it is processed and also retains its own qualities). This quality of Ghrita makes it an excellent vehicle for drug administration and its spreading nature facilitates the dispersion of all medicinal properties to the deepest tissues.

In this context it is also referred to as catalytic agent. Ayurveda has traditionally considered Ghrita to be the healthiest source of edible fat with many beneficial properties like improving voice and complexion, promoting memory, intelligence and power of digestion, boosting up immunity, helping in absorption of vital nutrients, lubricating the connective tissues there by rendering the body more flexible. Ayurveda described for the regular consumption of Ghrita as it boosted the mental as well as physical strength of the individual and also warded off diseases. Medicated Ghrita was given foremost importance [8].

Composition of Ghrita

Ghrita made from cow milk is considered as superior to all other Ghrita. Like many dairy products, Ghrita is composed almost entirely of fat, 62% of which consists of saturated fats. Ghrita is a valuable source of fat-soluble vitamins A, E, and K. It is a rapid source of energy compared to other vegetable oils

because it contains short-chain fatty acids (FAs), which are readily absorbed and metabolized. Ghrita belong to an important category of bio-organic compounds called as lipids, which are essential for living organisms, besides carbohydrates, proteins and nucleic acids. Lipids, a type of carbon containing organic compounds, are classified into saturated fatty acids (SFAs), that have no carbon-carbon double bonds in the carbon chain, and unsaturated fatty acids (UFAs), that have one or more than one carbon-carbon double bond in the carbon chain, based on their saturation levels. UFAs is further categorized as monounsaturated UFAs (MUFAs) and polyunsaturated UFAs (PUFAs). SFAs with the same number of carbons in the chain will have a higher melting point as compared to UFAs as the presence of double bonds lowers the melting point [9].

Shat dhaut ghrita (SDG)

SDG is very popular in Ayurvedic system of medicine which is having potential as a topical product for treatment of skin diseases; it needs evaluation in modern scientific parameters. Cream formulation is evaluated by checking its organoleptic properties, spreadability coefficient and extrudability. Cream formulation was tested for anti-inflammatory study in animal model and that was compared with marketed formulation containing synthetic drug. From the results, it was found that developed cream shows better (11.72%) inhibitions of paw volumes than the marketed one (7.80%). Results of the study reveal that formulated cream containing *Curcuma amada* in Shat dhaut ghrita could be a better formulation in treating inflammation topically compared to available topical formulations containing synthetic NSAIDs [10, 11].

Burn injuries are frequent, horrifying, and life-threatening conditions that are still challenging to cure. Complications like scarring, keloid formation, and contractures are the main challenges for the treating surgeon. The Ayurvedic classics explain different treatment modalities for the management of burn wounds, like oral medicines, topical applications prepared with herbal medicines, ghee, oil, and

typical wound dressing techniques like Patraadaana (wound covering with medicinal leaves), and fumigation therapy, etc.

Preparation of Shata-Dhauta-Ghrita

2.5 kg standardized cow ghee was taken in copper vessel. 1.5 L distilled water was added to it. The mixture of cow ghee and water was triturated for 5-8 min and contents in copper vessel were allowed to settle. Then water was decanted carefully, avoided loss of ghee. Then fresh 1.5 L distilled water was added in previously washed cow ghee and similar procedure was repeated one hundred times to obtain Shat-dhauta-ghrita [12].

GHRITA PHARMACOLOGICAL ACTION:

It contains approximately 8% lower saturated fatty acids, making it easily digestible. These are the most edible fats and are not found in any other edible oil or fat. It contains vitamins, including Vit A and Vit. E is anti -oxidants and is helpful in reducing ketone bodies, helpful in preventing oxidative injury to the body. Vit. A keeps epithelial tissue of the body intact keeps the outer lining of the eyeball moist and prevents blindness. Essential fatty acids promote proper growth of the human body. The melting point of Ghrita is 350C which is less than the normal temperatures of the human body. Its digestibility coefficient or rate of absorption is 96% which is highest of all oils and fats. Active ingredients are easily digested and absorbed because they are combined with Ghrita. Because cell membranes contain lipid, Ghrita's lipophilic action facilitates transportation to a target organ and final delivery within cells. Ghrita's Lipophilic action facilitates the formulation's entry into the cell and delivery to the mitochondria microsome and nuclear membrane. Ghrita contains beta-carotene and vitamin E, both of which are antioxidants [13].

Introduction to QbD

Quality by Design (QbD) is defined as a systematic approach to development that begins with

predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. The Quality related guidelines such as the "Systems and Risk based approaches to cGMPs" revealed new key words for industry introducing the concepts of QbD, Quality Risk Management (QRM), Pharmaceutical Quality Systems (PQS) etc [14]. Quality by Design (QbD) concept was first developed by Dr. Joseph M. Juran. His belief was that quality is something that has to be designed into a product and that the method of designinga product is responsible for most of the crises and problems associated with quality. The International Conference on Harmonization (ICH) now called Harmonization for Better Health has issued four guidelines i.e. ICH Q8 (R2), Q9, Q10, Q11 and Implementation documents that provide a general framework for the application of QbD to drug product and drug substance development and manufacture. In particular, ICH Q8 (R2) (issued in 2005 and revised in 2009) outlines and defines the main elements of the QbD framework. QbD tools facilitate a higher level of process understanding, root cause investigation [15].

2 REVIEW OF LITERATURE

The clinical efficacy of the Manjishthadi ghrita (trial drug) was evaluated for wound healing. The drug Manjishthadi Ghrita possesses high efficacy in vrana ropana with fine scaring without producing any adverse effect. So, it can be recommended as a cost-effective, easy to prepare and effective therapy for wound healing [16]. Cow ghee based Polyherbal Bhallatakadi Ghrita formulations and evaluation was prepared with reference to 'Murchhana' and 'Shata-Dhauta' process. They were subjected prepared ghrita formulations to organoleptic, evaluation, in-vitro antioxidant and GC-MS analysis. The accelerated and real time stability studies were carried out to determine shelf life of Ghrita formulations [17]. Yashtimadhu Ghrita in comparison with lignocaine–nifedipine ointment in the management of Parikartika (Acute fissure-in-ano) was studied. Both the interventions Yashtimadhu Ghrita and lignocaine–nifedipine ointment has equally effective in symptomatic relief in Parikartika (acute fissure-in-ano) [18].

An introduction and general overview on process validation of pharmaceutical manufacturing process with special reference to the requirements stipulated by the US Food and Drug Administration (FDA) of Solids (tablets and capsules), liquids and semisolids [19]. Ayurveda has mainly focused on important of the poly-herbalism and its clinical significance. It also state that Ayurveda is one of the traditional medicinal systems of Indian which involves the use of natural elements to eliminate the root cause of the disease by restoring balance [20]. *Curcuma amada* in Shat dhaut ghrita could be a better formulation in treating inflammation topically compared to available topical formulations containing synthetic NSAIDs [10].

Ghrita and its rasayana has analyzed in the management of dementia. Dementia is a chronic mental disorder, characterized by progressive, usually irreversible global cognitive deficit. The signs and symptoms along with pathogenesis of dementia can be understood in terms of smriti nasha, smriti

bhramsha [7]. Ghrita have analyzed for its organoleptic, physico-chemical features and screened for its phytoconstituents as well as its chromatographic analysis. Improvement of analytical methodologies can serve as a specific basis for research in herbal drug technology, thereby, facilitating the producers and researchers to set quality standards as well as parameters [15]. Phytoconstituents individually or in the combination, determine the therapeutic value of a medicinal plant. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes etc. are some of the important phytochemicals with diverse biological activities [21]. Shat-dhaut ghrita is an Ayurvedic preparation, commonly prescribed for treatment of wound, burns, chickenpox, scars, herps, leprosy, and other skin diseases and have potential for the treatment of skin diseases [22].

Sarasvata ghrita standardization was done by HPTLC method for determination of piperine, bebeerine6-shogaol and β- asarone and found the developed method was simple, specific, accurate, precise and robust and used for routine analysis [23]. Systematic review and analysis of the scientific research that carried out on the benefits and harms associated with the usage of Ghrita suggested beneficial effects of plain as well as medicated Ghrita on the various components of health and break the myth associated to the exclusion of Ghrita in diet. It also state that Ghrita consisted various fats (saturated, monosaturated and polyunsaturated), fatty acids, minerals, vitamins etc. And the composition of Ghrita varies along with the method of preparation [9].

Shat dhauta ghrita has potential as a topical product for treating skin diseases, but it must be evaluated using modern scientific parameters. SDG is act as an excellent cream base, if we incorporate herbal drug or phytoconstituent in the SDG. It act as a promising agent in the development of herbal cream [13]. Various treatment modalities for the management of burn wounds, like oral medicines, topical applications prepared with herbal medicines, ghee, oil, and typical wound dressing techniques like Patraadaana (wound covering with medicinal leaves), and fumigation therapy etc [11].

Pharmaceutical QbD designs and tools

1. Plackett-Burman (PB) design
2. Central composite design
3. Face centered design
4. Box-Behken design
5. Star designs
6. Mixture designs
7. Taguchi designs
8. Optimal designs

In view of above, present study is envisaged to screen any above or phytoconstituent with ghee for its effectiveness in pharmacological activities and formulate it in topical dosage form by using any above or any other suitable QbD design.

3. IMPORTANCE AND NEED OF STUDY

Now a day's people are more cautious about their health and demanding certain products that are closely associated with nature so use of herbal product is increasing tremendously for treating various diseases and disorders. semisolid dosage forms intended for topical application such as creams, ointments, gels, and lotions are prepared using synthetic bases have numerous disadvantages. Natural bases, often derived from plant or animal sources, offer distinct advantages in these formulations. natural base such as Shat Dhaut Ghrita (SDG) have been used in topical traditional medicine for generations. Incorporating therapeutic phytoconstituents into Shat Dhaut Ghrita (SDG) Ayurvedic semisolid system can align with cultural practices and indigenous knowledge.

In the proposed work our intent is to come up with modern Pharmaceutical dosage form using Shat Dhaut Ghrita (Ayurvedic / Traditional semisolid system) by QbD Approach and to evaluate its effectiveness and stability by studying modern scientific parameters. The Developed topical dosage form will be improve patient compliance and enhance effectiveness of treatment

4. OBJECTIVES

- Characterization of ghee using HR-LCMS method.
- Carryout molecular docking of various known phytoconstituents.
- Formulate shat dhaut ghrita based formulations using phytopharmaceuticals.
- Evaluate Pharmaceutical parameters of prepared Topical formulations
- Optimization of prepared Topical formulation using QbD approach
- Evaluation of optimized formulation using *in-vitro* Diffusion study.
- Evaluation of Pharmacological activity of optimized formulation.

5. HYPOTHESIS

- By taking idea from literature review we are decided to work on the preparation of Shat dhaut ghrita based formulation by using QbD approach.
- Due to long term treatment for skin related problems, the Ghrita formulation therapy can be the best alternative over synthetic drugs to minimize the occurrence of side effects.
- The purpose of the study is to examine such potent phytoconstituents and creating Shat Dhaut ghrit formulations by overcoming the all problems which will be helpful in the management of skin related disorders.

6. METHODOLOGY

I. **Collection and Identification of Ghee:**

Ghee will be collected from the known sources and identified

II. **Characterization and Selection of Ghee:** All collected ghee samples will be characterized and their lipid constituents are determined by HR-LCMS and will be selected based on same.

III. **Formulation of Shat- Dhaut Ghrita**: It will be used as formulation base for topical dosage form.

IV. **Evaluation of Shat- Dhaut ghrita** :

V. **Collection and selection of phytoconstituents:** Phytoconstituents will be collected and selected according to its pharmacological activity by using molecular docking.

VI. **Compatibility Study:**

VII. **Pre-formulation studies**

VIII. **Formulation of topical dosage form using (SDG)**: It will be developed by QbD approach.

IX. **Optimization of formulation:** QbD approach will be applied to the observed evaluation parameters for optimization process.

X. **Evaluation of optimized formulation by *in vitro*-Diffusion and *in vivo* evaluation using animal model.**

XI. **Stability study of Optimized formulation.**

XII. **Compilation of data, Statistical Analysis, Interpretation of observations and Conclusions**.

7. DURATIONOFWORK

Sr.No.	Work	Period
1.	Literature survey	6 months
2.	Collection of different types of ghee and identify lipid constituent by HP-LCMS	2 months
3.	Phytochemical study	3 months
4.	Selection of specific phytoconstituent by using molecular docking	3 months
5.	Pre-formulation study of Shat Dhaut ghrita.	1 months
6.	Formulation & development of Topical Dosage by QbD Approach.	6 months
7.	Pharmaceutical Evaluation of formulated Shat Dhaut Ghrita.	6 months
8	Evaluation of formulated product for its pharmacological activity using *in-vitro* and *in-vivo* test.	6 months
9.	Other work, Compilation of data, Interpretation and Conclusion	4 months
	Total duration of work	36 months

8. REFERENCES

1. Kawade DP, Gulkari VD, Nakhate YD, Jain NN. Formulation and physicochemical evaluations of polyherbal ghrita. World journal of pharmaceutical research, 2019; 8(5): 1205-1226
2. Ramaiah M, Chakravathi G, Yasaswini K. In vitro biological standardization, formulation and evaluation of directly compressed polyherbal anthelmintic tablets. Pharmacognosy Journal, 2013; 5(3):130-134.
3. Saraf S. Evaluation of physicochemical and phytochemical properties of Safoof-E-Sana: A Unani polyherbal formulation. Pharmacognosy research, 2010; 2(5): 318.
4. Reddy MS, Reddy DR, Prasad NA. Herbal and pharmaceutical drugs enhanced with probiotics. 2000. United States Patent, Patent Number: 6,080,401, Date of Patent: Jun. 27, 2000
5. Jayaweera JA. Introduction to Ayurvedic Formulations: Exploring the Classical Concepts with Modern Science.
6. Angadi SS. Management of Acne Vulgaris: An Ayurvedic Approach. Indian Journal of Ancient Medicine and Yoga, 5500 (5000): 95.
7. Nambiar S, Nidhin MM, Kadibagil VR. Critical Review on Management of Dementia with Ghrita Kalpana. World J Pharm Res., 2017; 6:316-23.
8. Jayaweera JA. Introduction to Ayurvedic Formulations: Exploring the Classical Concepts with Modern Science.
9. Singh VV, Rai VS. A systematical review of traditional Ayurvedic and morden medical perspectives on Ghrita (clarified butter): a boon or bane. Traditional Medicine Research. 2019; 4(6): 29.

10. Majumdar SH, Kulkarni AS, Aloorkar NH, Shinde MD. Exploring shat dhaut ghrita as formulation base for the topical delivery of Curcuma amada. Indo American Journal of Pharmaceutical Research, 2016; 6(7):pageno

11. Shindhe PS, Priyanka K, Killedar RS, Prasannan D, Kirthi A. An integrated management (Ayurveda and Modern medicine) of accidental burn injury: A case study. Journal of Ayurveda and integrative medicine. 2023; 14(2): 100691.

12. Majumdar SH, Kulkarni AS, Aloorkar NH, Shinde MD. Exploring shat dhaut ghrita as formulation base for the topical delivery of curcuma amada. Indo American Journal of Pharmaceutical Research, 2016, ISSN NO: 2231-6876

13. Deshmukh MD, Patil MP, Ahire ED, Gosavi SB. Shatdhauta Ghrita: A Promising agent in the development of herbal creams. Journal of Pharmaceutical Negative Results, 2022: 1332-43.

14. Buttini F, Rozou S, Rossi A, Zoumpliou V, Rekkas DM. The application of quality by design framework in the pharmaceutical development of dry powder inhalers. European Journal of Pharmaceutical Sciences, 2018; 113: 64-76.

15. Mishra V, Thakur S, Patil A, Shukla A. Quality by design (QbD) approaches in current pharmaceutical set-up. Expert opinion on drug delivery, 2018; 15(8): 737-58.

16. Baria J, Gupta SK, Bhuyan C. Clinical study of Manjishthadi Ghrita in vrana ropana. Clinical Research, 2011; 32(1): pageno-1-5.

17. Sandesh R. Wayal A, Gurav SS. Bhallatakadi Ghrita: Development and evaluation with reference to Murcchana and Shata-Dhauta process. Journal of Ayurveda and Integrative Medicine, 2020; vol 11 (2020) 261-269.

18. Patel JR, Dudhamal TS. A comparative clinical study of Yashtimadhu Ghrita and lignocaine-nifedipine ointment in the management of Parikartika (acute fissure-in-ano). AYU, 2017; 38(1-2): page no1-51.

19. Panday P, Bharadwaj R, Chen X. Modelling of drug product manufacturing processes in the pharmaceutical industry. Predictive modelling of pharmaceutical unit operation, 2017: 1-13.

20. Parasuraman S, Thing GS, Sokkalingam AD. Polyherbal formulation: Concept of Ayurveda, Pharmacognosy review, 2014; 8(16): 73.

21. Shaikh JR, Patil MK. Qualitative test for preliminary phytochemical screening: An overview, International Journal of Chemical Studies, 2020; 8(2): 603-608.

22. Deshpande S, Deshpande A, Tupkari S, Agnihotri A. Shata-dhauta ghrita- Case study, CSIR,2009.

23. Shelar M, Lohidasan S, Gadgil S, Mahadik KR. Marker based standardization of Saraswata ghrita, an Ayurvedic Polyherbal formulation using Rapid validated HPTLC Method. J. Chem. Pharm. Res., 2017 9(10): 1.

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